MEAT ANALYSIS

Rapid Determination of Fat in Meat Products

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Determinations of fat in meat products require approximately 30 minutes with a modified Babcock procedure. Clear fat columns are separated with a perchloric acid–acetic acid mixture which completely digests proteins, cereals, and spices. Results agree closely with those obtained by the dry solvent extraction technique of the Association of Official Agricultural Chemists.

THE GREAT IMPORTANCE of meat products for feeding the Armed Forces demands close attention to those factors which affect quality. Effective control of fat content, and hence of price, is especially important under the system of competitive bidding by which the Armed Forces procure food supplies. Limiting the amount of fat for improved acceptability is even more important for reasons of morale, nutrition, and economy, and because disposal of surplus fat is difficult under certain combat conditions. The fat content is one of the significant quality factors which can be measured objectively, and this paper reports a rapid control method for determining the percentage of fat in meat products.

A number of laboratory methods can provide accurate knowledge of the fat content of meat products. Among these, the official method of the Association of Official Agricultural Chemists (*3*) is the most widely accepted. The results by that procedure, which involves solvent extraction of the dried material, are normally obtained on the third day after starting the analysis. Solvent extraction of the wet material following acidification (*4*, *25*) or acid hydrolysis (*27*) is a shorter procedure, but the number of man-hours of laboratory work involved is as great, if not greater, than that required by the AOAC method. Although these methods are useful for evaluating the finished products, they obviously cannot be used for controlling the manufacturing process. The results of control tests must be available in a matter of minutes, rather than hours or days, in order to permit formula adjustments before the products are frozen, canned, or otherwise processed or packaged. Such control would facilitate compliance with military specifications and should result in fewer rejections, lower cost, and improved acceptability.

More rapid methods for measuring the fat content of meat utilize principles other than the gravimetric determination of the isolated fat. Some are based upon the changes in physical properties of a solvent used for extracting the fat. Harris (*8*) and Herty, Stem, and Orr (*9*) measured the specific gravity of the fat-solvent extract. The method of Matrozova (*13*) is based on the refractive index of the extract. Furgal (*5*) described a method for measuring the high-frequency impedance of the fat solution. Volumetric methods have been adapted from procedures used on dairy products. Copeland (*10*) digested the meat with a modified Minnesota reagent (*1*) containing sodium salicylate, potassium carbonate, sodium hydroxide, and isopropyl alcohol and measured the volume of the separated fat in a Babcock cream bottle. Oesting and Kaufman (*16*) liberated the fat from an emulsified sample with acetic and sulfuric acids in a Babcock milk test bottle. Talbot (*24*) used sulfuric acid and amyl alcohol in a butyrometer. Oesting and Kaufman and Talbot discussed the interference with their methods caused by cereals. More recently, Kelley, Guerrant, and Mackintosh (*11*) reported on several volumetric methods and obtained good results on ground beef by digesting it with acetic and sulfuric acids in a Paley-type Babcock cheese bottle. They found that method unsatisfactory, however, for pork sausage containing seasoning.

For use in connection with military procurements, the Quatermaster Corps requires a method for fat which is rapid, reliable, applicable to a variety of meat products, and adaptable to use by a traveling inspector in locations lacking normal laboratory facilities. It was desirable to avoid the use of toxic or flammable solvents or very specialized equipment. The modified Babcock procedure described here is similar to others which have been reported, but it has the distinct advantage of being free of interference from cereals and seasoning.

Method

Materials Food chopper equipped with plate with $^5/_{64}$-inch openings; or Waring or similar food blender.

Torsion balance.

Centrifuge or Babcock tester, unheated.

Metal beaker or bath.

Paley-type Babcock cheese bottles, 20% and 50% size (Kimble Glass No. 508 and 509), with rubber stoppers.

Medicine dropper, preferably USP official medicine dropper (Glasco Products No. 2020).

Dividers.

Perchloric acid–acetic acid mixture, prepared by mixing equal volumes of reagent grade glacial acetic acid and reagent grade perchloric acid of 60% strength.

Glymol, or red mineral oil, specific gravity approximately 0.82 at 20° C. (Kimble Glass No. 730).

Procedure The sample should be at or near room temperature. Pass it rapidly through a food chopper three times and mix thoroughly after each grinding. Alternatively, comminute the sample in a food blender. Avoid prolonged blending, which causes overheating of the sample. In the case of canned meats, prepare the entire contents of the can.

Weigh 9.00 grams of the prepared sample into a tared Paley-type Babcock bottle of either 20 or 50% size, depending upon the fat content of the sample. Use a 20% bottle when the fat content is not more than 15%; otherwise, use a 50% bottle. If the fat content is more than 45%, or if it is desired to halve the time required for digestion, use 4.50 grams of sample and multiply the final reading

Reprinted from **AGRICULTURAL AND FOOD CHEMISTRY,** Vol. 3, No. 7, Page 588, July 1955

by 2. Add 30 ml. of the perchloric acid–acetic acid mixture, stopper the Paley bottle, and swirl it to mix the contents. Immerse the bottle in a boiling water bath and agitate occasionally during the heating period until the sample is completely digested. Approximately 12 minutes are required to digest 9 grams of material. A metal bath, such as a stainless steel beaker, should be used. Observe the usual precautions in the handling and storage of perchloric acid (*12*).

It is always well to consider the conditions under which the use of perchloric acid is regarded as safe or hazardous. After 30 ml. of the acid mixture have been added to 9 grams of sample, the perchloric acid–water weight ratio is reduced to approximately 1 to 1, or a molar ratio of approximately 1 to 6. Based on information available in the literature, the concentration and temperature encountered in the test are well within safe limits. According to Smith (*22*) hot dilute perchloric acid is not an oxidizing agent. In discussing the perchloric acid–acetic acid mixture used in their modified Babcock test for ice cream, Smith, Fritz, and Pyenson (*23*) state that such mixtures are not hazardous to mix and to store and that the method requires no precautions other than those applied to the unmodified Babcock test.

Remove the bottle from the bath as soon as digestion is complete and add more acid mixture until the fat column rises into the calibrated neck of the bottle. Centrifuge the bottle, after careful balancing, for 2 minutes at recommended Babcock speed (875 r.p.m. at 15-inch diameter). If, after centrifuging, the fat column extends below the zero mark in the calibrated neck of the bottle, add more of the acid mixture and centrifuge again for 1 minute.

With the aid of a pair of dividers, measure the length of the fat column as quickly as possible after centrifugation is completed. When using the 20% bottle, if the reading is greater than 11.0, add 1 drop of colored glymol from a medicine dropper before making the final measurement. With the 50% bottle, always add 3 drops of glymol before measuring the fat column. Add the glymol so that it flows gently down the inside wall of the neck. Fat columns without glymol should be measured from the lowest to the highest point (including meniscus). Fat columns with glymol should be measured from the lowest point to the level interface between the fat and colored glymol. The final reading represents the percentage of fat when a 9-gram sample is used.

Experimental

Because of the advantages for the purpose intended, greatest emphasis was placed on the Babcock method. The record of performance and acceptance of the Babcock test in the dairy industry influenced this decision. The Paley-type cheese bottle was adopted because of its convenient design. A variety of reagent combinations were tried and the following were discarded, either because of incomplete recovery of fat or because a curd of undigested material interfered with the measurement of the fat column.

Sulfuric acid and glacial acetic acid (*15*)
Sulfuric acid, glacial acetic acid, and Oakite (*16*)
Sulfuric acid and *n*-butyl alcohol (*14*)
Sulfuric acid, hydrochloric acid, and *n*-butyl alcohol (*18*)
Sulfuric acid and potassium persulfate
Sulfuric acid and potassium dichromate
Sulfuric acid and hydrogen peroxide
Hydrochloric acid and hydrogen peroxide
Sodium salicylate, potassium carbonate, sodium hydroxide, and isopropyl alcohol (modified Minnesota reagent) (*1*)
Ammonium hydroxide, *n*-butyl alcohol, ethyl alcohol, trisodium phosphate, and sodium acetate (Illinois reagent) (*17*)
Combinations of anionic and nonionic detergents (*6, 7, 21*)
Combinations of anionic and nonionic detergents with sodium chloride (*19*)
Nonionic detergent Triton X-100, sodium tetraphosphate, and methanol (*20*)

In addition to these mixtures, combinations of sulfuric acid and water were thoroughly investigated. Satisfactory results were obtained on ground beef, canned beef with gravy, and canned pork and gravy when they were digested with sulfuric acid. However, excessive charring of the samples and suspended curds of undigested material frequently interfered with accurate measurement of the fat columns. In order to avoid these difficulties it was necessary to use a different set of test conditions for each product. Proper adjustment of the amounts of sample, water, and acid became a matter of delicate balance difficult to accomplish in all cases.

The quantitative separation of butterfat from dairy products with various detergent mixtures has been reported by a number of investigators (*6, 7, 19, 20, 21*). When these mixtures were applied to meats in this study, clear fat columns were separated but recoveries were never quantitative. Even when recoveries appeared complete (101 to 104% of the AOAC value), approximately 20% more fat was recovered from the insoluble residue with the perchloric acid–acetic acid mixture described in the method. Solution of detergent in the fat or the formation of a stable detergent-fat complex was apparently responsible for the expanded volume of the separated fat.

Smith, Fritz, and Pyenson (*23*) reported the use of perchloric and acetic acids in a modified Babcock test for ice cream. They claimed the following advantages for their reagent, which consisted of a mixture of equal parts by volume of 72% perchloric acid and glacial acetic acid.

No interference by sugars and flavors
Charring action of sulfuric acid avoided
Sugar and proteins soluble in the reagent
Fewer operative details (one reagent, one centrifugation)
Reagent concentration not critical
Stable acid mixture; no special precautions
Test bottles cleaned with hot water alone

The principal modification made in the procedure of Smith, Fritz, and Pyenson was to substitute 60% perchloric acid for 72% perchloric acid. With the more concentrated acid the fat columns separated from low-fat samples were often dark and difficult to measure. The 60% acid performed satisfactorily in all cases and, incidentally, costs less. The modified reagent was used on a variety of meat products with good results. Approximately 30 minutes are required for four determinations. The claims made for the acid mixture were verified and the difficulties encountered with other reagents were avoided. The samples were always completely digested and the fat columns were light colored, clear, and free of curd or foam. Cereals and seasoning caused no interference (Figure 1).

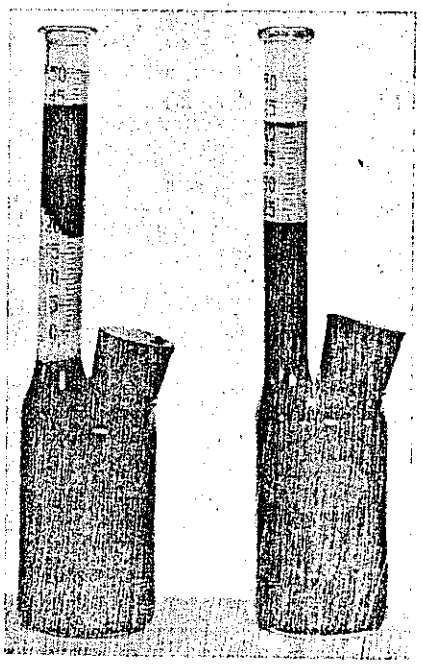

Figure 1. Babcock test on canned pork and gravy

Left. Digested with sulfuric acid; note suspended curd of undigested material
Right. Digested with perchloric-acetic acid mixture

Inasmuch as the AOAC method (*1*) is official for military specifications, the conditions of the rapid test were selected in such a manner as to bring the two methods into agreement. The rapid method usually gave higher results than the AOAC method, but these were re-

duced to the desired values by depressing the upper meniscuses of the fat columns with glymol—1 drop to the 20% size Babcock bottle for samples containing more than 11% fat, and 3 drops to the 50% bottle. Glymol is used also in many of the Babcock procedures for dairy products.

In following the AOAC method the samples were dried at 101° C. for 16 hours and then extracted in a Soxhlet apparatus with petroleum ether. Military specifications have for several years prescribed the use of petroleum ether (boiling range 30° to 60° C.) in place of anhydrous ethyl ether for the extraction of fat from meats. Comparative tests of the two solvents were made on ground beef and the results confirmed Windham's (27) finding of no significant difference.

The effects of varying the specific gravity of the glymol and the size and number of drops used were investigated. Red glymol of specific gravity 0.82 at 20° C. was used in this work. Comparisons were made in tests on ground beef with other mineral oils of specific gravity 0.83 and 0.84 at 20° C. The heavier oils were colored red by adding Sudan IV and filtering through a fritted-glass funnel of medium porosity. The variation in specific gravity from 0.82 to 0.84

Table I. Determinations of Fat in Meat and Meat Products

Sample	Conditions of Babcock Test: Sample weight, g.	Conditions of Babcock Test: Bottle size, %	Fat Content, %: AOAC	Fat Content, %: Babcock
1. Ground beef[a]	9.0	50	30.05, 30.09	30.0, 30.0, 30.1, 30.5
			Av. 30.07	30.15
2. Ground beef[a]	4.5	20	22.34, 22.46, 22.60	22.4, 22.4, 22.4, 22.6
	9.0	50		22.8, 22.8, 22.9, 23.0
			Av. 22.47	22.66
3. Ground beef[a]	4.5	20	29.02, 29.22	28.6, 28.8, 28.8, 29.0
	9.0	50		29.0, 29.0, 29.1, 29.2, 29.3, 29.5
			Av. 29.12	29.03
4. Ground beef[b]	4.5	20	13.60, 13.62, 13.65	13.2, 13.2, 13.2, 13.2
	9.0	20		13.2, 13.4, 13.5, 13.6
	9.0	50		13.3, 13.4, 13.4, 13.5
			Av. 13.62	13.34
5. Pork[c]	4.5	20	15.23, 15.41, 15.64	15.4, 15.4, 15.4, 15.4, 15.6, 15.6
	9.0	20		15.1, 15.4, 15.6, 15.6
	9.0	50		15.0, 15.5, 15.5, 15.6
			Av. 15.43	15.44
6. Pork[c]	4.5	20	16.99, 17.03	16.6, 17.0, 17.0, 17.0
	9.0	50		16.8, 16.9, 17.0 17.1
			Av. 17.01	16.93
7. Veal	4.5	20	16.63, 16.63, 17.19	17.0, 17.2
	9.0	50		16.6, 16.7, 16.7, 16.7, 16.8, 16.8
			Av. 16.82	16.81
8. Veal	4.5	20	27.02, 27.43	26.8, 27.0
			Av. 27.23	26.90
9. Veal fat	4.5	50	83.54, 84.09	83.6, 84.0, 84.2, 84.2
			Av. 83.82	84.00
Meat Products				
10. Beef with gravy, canned	4.5	20	11.95, 12.00	11.6, 11.6, 11.6, 11.8
			Av. 11.98	11.65
11. Beef with gravy, canned[d]	4.5	20	9.94, 9.96	10.4, 10.4, 10.4, 10.4
			Av. 9.95	10.40
12. Beef with gravy, canned[d]	4.5	20	4.04, 4.05	4.2, 4.4, 4.4, 4.4
	9.0	20		3.8, 3.9, 3.9, 3.9
			Av. 4.05	4.11
13. Beef with gravy, canned[d]	4.5	20	6.41, 6.54, 6.63	6.8, 6.8, 7.0, 7.0
	9.0	20		6.2, 6.3, 6.6, 6.6
	9.0	50		5.9, 6.0, 6.0, 6.0
			Av. 6.53	6.66
14. Pork and gravy, canned[e]	4.5	20	10.26, 10.29	10.4, 10.4, 10.4, 10.4
	9.0	20		10.2, 10.2, 10.3, 10.4
			Av. 10.28	10.34
15. Pork and gravy, canned[e]	4.5	20	18.53, 18.82	18.6, 18.6, 18.6, 18.6
			Av. 18.68	18.60
16. Pork and gravy, canned[e]	4.5	20	12.19, 12.38, 12.50	12.0, 12.0, 12.2, 12.4
	9.0	20		12.3, 12.4, 12.4, 12.4
	9.0	50		12.1, 12.2, 12.3, 12.4, 12.4
			Av. 12.36	12.27
17. Veal loaf, raw[f]	9.0	50	21.30, 21.36, 21.90	21.4, 21.5, 21.5, 21.5
			Av. 21.52	21.48
18. Veal loaf, baked[f]	4.5	20	15.71, 16.12, 16.13	15.6, 15.8
	9.0	50		15.5, 15.6, 15.6, 15.6, 15.6, 15.8
			Av. 15.99	15.64
19. Beans with frankfurter chunks in tomato sauce, canned[g]	4.5	20	9.54, 9.60	9.8, 9.8
	9.0	20		9.8, 9.8, 9.8, 9.9
	9.0	50		9.5, 9.6, 9.7
			Av. 9.57	9.82

[a] Derived from certain cuts and trimmings of choice grade carcasses. Military specification MIL-B-10017B.
[b] From primal cuts of utility or lower grade carcasses. Military specification MIL-B-723A.
[c] Picnics, hams, loins. Military specification MIL-P-1044B.
[d] Contains beef, flour, salt, pepper, caramel. Military specification MIL-B-723A.
[e] Contains pork, flour, salt, pepper, caramel. Military specification MIL-P-1044B.
[f] Contains veal, salt, onion powder, pepper, monosodium glutamate, cracker meal.
[g] Contains frankfurters, beans, tomato sauce, sugar, salt, onions, allspice, cinnamon, cloves, mace. Military specification MIL-B-1065A.

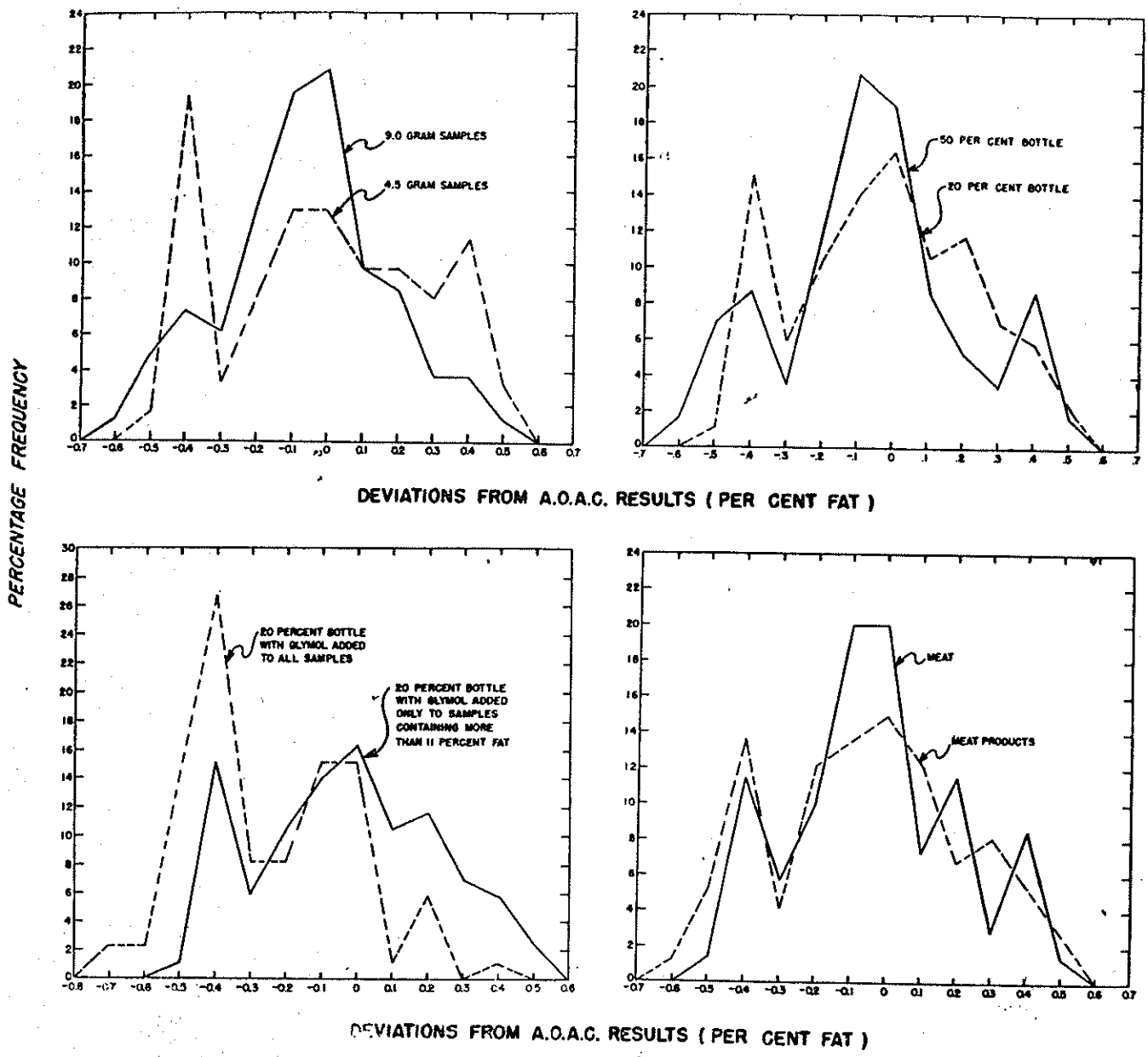

Figure 2. Frequency of stated deviations from AOAC results

did not produce any noticeable effect upon the results.

In order to measure the effect of variations in droppers, 12 USP official medicine droppers (*26*) were tested and the two droppers differing most widely in delivery rate were selected. One of these conformed to the USP specification and the second deviated by 10%. In tests on ground beef the results were the same with either dropper. It is important to add the correct number of drops of glymol. The quantities specified in the procedure were selected after numerous tests without glymol and with successive additions up to 3 drops.

The conditions of centrifugation were found to be not critical. With a centrifuge head 15 inches in diameter, results were the same when speeds of 400, 800, and 1200 r.p.m. were used for 2 minutes and when periods of 1, 2, and 4 minutes were used at 800 r.p.m.

Results and Discussion

Ten varieties of beef, veal, and pork products were tested by the rapid method and also by the AOAC method. Out of a total of 144 determinations by the rapid method 143 agreed with the AOAC method within ±0.5% fat. The results of all determinations are shown in Table I. The standard deviation of a single determination by the rapid method is 0.209% fat, and by the AOAC method is 0.196% fat. The average difference between duplicates is estimated as 0.236% fat for the rapid method, and 0.221% fat by the AOAC method. The standard deviations were computed by the method described by Youden (*28*). In order to arrive at a single standard deviation for the rapid method, all results for each sample were pooled without regard to the differences in test conditions. This was justified by examining the variances due to the different test conditions and finding them to be homogeneous.

Many of the data in Table I were gathered in the course of developing the rapid method, so that some of the test conditions described differ slightly from those finally selected. An example is the use of a 50% bottle for sample 4, which contains less than 15% fat.

Comparison with AOAC Method For each of the 19 samples tested the deviations of all results by the rapid method from the average AOAC value were determined. The frequency polygons in Figure 2 are a graphical presentation of these differences. The first graph demonstrates the advantage of using a 9-gram rather than a 4.5-gram sample. With the half-size samples 42% of the deviations were greater than ±0.3% fat, compared with only 18% for the larger samples. The

second graph shows that the bottle size had little effect on the distribution of deviations in fat percentages. Results with the 20% bottle on samples containing 11% or less of fat agreed most closely with AOAC results when no glymol was used. The third graph shows the beneficial alteration in the frequency distribution which resulted from the omission of glymol in those instances. The fourth graph compares meat with meat products containing cereals and seasoning. The frequency distribution is only slightly less favorable for the meat products.

oxidation and larger unsaponifiable residues. In a similar comparison in extraction of fat from liver, Bixby, Bosch, Elvehjem, and Swanson (*4*) obtained even greater differences and presented evidence suggesting that the values obtained by dry ether extraction were too low.

A few comparisons were made on ground beef between the AOAC method and the acid hydrolysis method formerly used in military specifications (*27*). Results by the acid hydrolysis procedure were higher by approximately 0.3% fat.

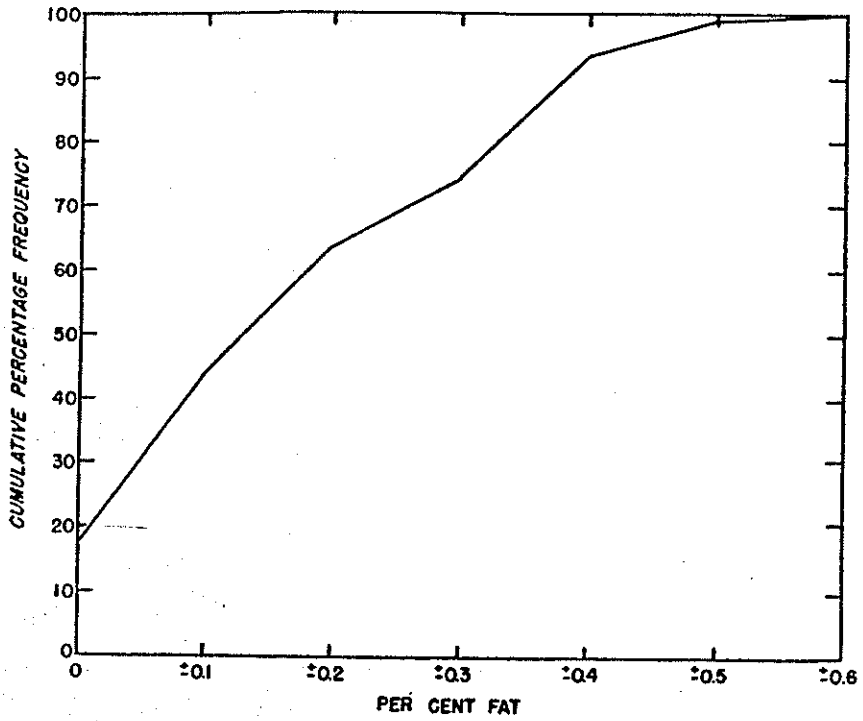

Figure 3. Percentages of determinations agreeing with AOAC results within stated per cent fat

Figure 3 is a cumulative frequency distribution of the differences in results by the AOAC and rapid method. Less than 1% of the deviations exceeded 0.5% fat. The size and direction of the deviations from the AOAC results were independent of the fat content of the samples.

Agreement of the rapid method with the Soxhlet extraction method of the AOAC was sought for conventional reasons, but it has not been established beyond all doubt that the extract obtained with ether or petroleum ether represents the true fat content of the sample. The fat contents of many foods are determined by solvent extraction following acid hydrolysis. The AOAC (*2*) describes such methods for fish, eggs, cereals, and other products. Windham (*27*) averaged approximately 0.4% more fat in ground beef by an acid hydrolysis procedure than by dry solvent extraction in a Soxhlet apparatus. He indicated that the higher values by acid hydrolysis may be due in part to fat

Applications of Rapid Method

The rapid method was used to control a pilot scale preparation of 32 pounds of oven-ready veal loaf. A recent military specification for frozen veal describes the product and limits the average fat content to 22%. Veal and veal fat were passed through a meat grinder and then analyzed by the rapid method (samples 7 and 9, Table I). Based on these analyses, appropriate portions of meat, fat, cracker meal, and seasoning were blended together. The fat content of the final mixture (sample 17, Table I) was found to be 21.5%, by both the rapid and the AOAC methods. This close adherence to the specification requirement was made possible by the accurate and timely knowledge of the fat content of the ingredients which the rapid method provided.

The modified perchloric acid–acetic acid Babcock test described here requires less skill and less time than do the solvent extraction procedures. Although it is intended as a practical control test for the processing of meat products, it should be applicable to the laboratory analysis of a variety of foods.

Acknowledgment

The authors thank Clarence Sidwell for his assistance in reviewing the literature and for his helpful suggestions.

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Received for review March 29, 1955. Accepted April 26, 1955. Presented before the Division of Agricultural and Food Chemistry at the 127th Meeting of the AMERICAN CHEMICAL SOCIETY, *Cincinnati, Ohio, 1955. Research undertaken by the Quartermaster Food and Container Institute for the Armed Forces. No. 506 in series of papers approved for publication. The views or conclusions are those of the authors and are not to be construed as necessarily reflecting the views or endorsement of the Department of Defense. The mention of commercial products does not imply that they are endorsed or recommended by the Department of Defense over similar products not mentioned.*

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